

# Final Abstract

Final Report Approved on December 4, 2024

## M.L. 2020 Project Abstract

For the Period Ending June 30, 2024

**Project Title:** Freshwater Sponges And AIS: Engaging Citizen Scientists

**Project Manager:** Venugopal Mukku

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**Website:** <https://www.crk.umn.edu/>

**Funding Source:**

**Fiscal Year:**

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 03k

**Appropriation Amount:** \$400,000

**Amount Spent:** \$298,290

**Amount Remaining:** \$101,710

### Sound bite of Project Outcomes and Results

The project elicited enthusiastic participation from citizen scientists. A species unreported from Minnesota was identified. Gas Chromatography-Mass Spectrometry analysis of sponge extracts led to an intriguing discovery. 1,3,5-triphenylcyclohexane, sometimes detected in packaged foods, was detected in the extracts of about 40 sponge specimens collected from different places.

### Overall Project Outcome and Results

Freshwater sponges belong to the Phylum Porifera. Very little is known about their diversity and distribution in Minnesota's lakes and rivers. The study team wanted to study their diversity, distribution, taxonomy, and chemistry in Minnesota. With the advances in genetics, the study team wanted to combine traditional morphological and spicule analysis with genetic analysis to determine the taxonomy.

The land of 10,000 lakes presents a huge opportunity and a challenge to explore all water bodies comprehensively. So, the project team enlisted the participation of citizen scientists. Over the last few years, the team visited various science fairs, including the state fair. Their booth was always full of eager, curious children and parents.

The project has made significant strides toward achieving the originally stated outcomes, particularly in increasing public

awareness and engagement with Minnesota's freshwater sponges. Over the course of the project, 194 sponge collection kits were distributed to citizens across the state, leading to 41 kits being returned with samples. These efforts have resulted in the identification of eight distinct species of freshwater sponges, including three that were not previously reported in Minnesota.

The dissemination of project results has focused on sharing findings through various channels. The team has engaged with the public through county fairs, where citizens were educated about freshwater sponges and provided with collection kits. Additionally, a freshwater sponge collection site was created on CitSci.org, allowing for broader citizen participation and data sharing. Data and information were also shared with the Extension Office and MN DNR.

An unanticipated outcome of the chemical analysis of sponge extracts is the discovery of 1,3,5-triphenylcyclohexane in multiple sponge extracts. Very little is known about this chemical, although a few studies have been reported. In the future, we would like to study the extent of this chemical in

### **Project Results Use and Dissemination**

Project results were disseminated at multiple conferences and DNR meetings and included in the final report. The taxonomic data of the sponges will be published later. Identifying plastic leachate, the chemical 1,3,5-triphenylcyclohexane, from sponges collected at diverse locations is most intriguing and needs a deeper study. A future LCCMR project will be proposed to study the spread of this chemical in Minnesota's waters.



## Environment and Natural Resources Trust Fund

M.L. 2020 Approved Final Report

### General Information

**Date:** December 30, 2024

**ID Number:** 2020-027

**Staff Lead:** Noah Fribley

**Project Title:** Freshwater Sponges And AIS: Engaging Citizen Scientists

**Project Budget:** \$400,000

### Project Manager Information

**Name:** Venugopal Mukku

**Organization:** U of MN - Crookston

**Office Telephone:** (218) 281-8097

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### Project Reporting

**Final Report Approved:** December 4, 2024

**Reporting Status:** Project Completed

**Date of Last Action:** December 4, 2024

**Project Completion:** June 30, 2024

### Legal Information

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 5, Sec. 2, Subd. 03k

**Appropriation Language:** \$400,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota, Crookston, to use citizen scientists to study the geographic distribution, taxonomic diversity, and antifouling potential of freshwater sponges against aquatic invasive species.

**Appropriation End Date:** June 30, 2024

## Narrative

**Project Summary:** Freshwater sponges from Minnesota will be collected using citizen scientists thereby stimulating STEM education. Compounds produced by sponges will be tested against invasive species such as zebra mussels.

**Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.**

Sponges are among the most ancient living basal Metazoa and grow both in marine and freshwater environments. They are sessile animals and play a significant role in aquatic communities as filter feeders. Because freshwater sponges are sessile animals, they are also known to produce interesting chemical compounds that provide the sponge a chemical defense against other organisms. Despite the abundance of lakes and rivers in Minnesota, very few studies have been conducted examining the biogeographic distribution nor the chemistry of freshwater sponges in Minnesota. Sponges described in literature up to the 1970s and earlier relied heavily on morphological analysis in determining the taxonomy. Our limited exploration of MN lakes and rivers with funding from LCCMR (2017-2020) resulted in the discovery of two undocumented species in MN. Further, given the advances in taxonomic analysis and characterization, all new and known sponge species will be described by both morphological and molecular analysis. This will enable us to describe the phylogenetic relationships between various species.

Aquatic invasive species are a continued concern in the state of MN. Our preliminary research also showed that a few sponges contain antifouling chemicals that may be able to stop the spread of aquatic invasive species.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

One unanticipated and welcome outcome of the previous funding was the keen interest the project generated among high school students and ordinary citizens. We aim to capitalize on this interest in expanding our research statewide with the help of our undergraduate students, Master Naturalists and the University of Minnesota Center for Citizen Science. Involving citizens will enable us to sample multiple locations in all ten watersheds of the state in a short term (2021-2024). Sampling for sponges will occur in a narrow season (June to October).

We hope to collect 300- 500 sponge specimens. Many of those specimens may be identical species but that would enable us to determine the distribution and to compare the chemical composition of the same species from different lakes and rivers. We would prepare organic extracts of the collected specimens and test those extracts (where possible) on the growth of invasive species such as zebra mussels.

**What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?**

The project focuses on generating foundational data regarding the diversity and distribution of freshwater sponges in the state. The project involves citizen scientists and high school students and will be disseminated widely. Therefore, the public will become aware of the key role freshwater sponges play in the aquatic ecosystem. Public will also become aware of the interactions and competition using natural compounds between organisms. All data generated will be freely shared with MN DNR for dissemination to the public.

## Project Location

**What is the best scale for describing where your work will take place?**

Statewide

**What is the best scale to describe the area impacted by your work?**

Statewide

**When will the work impact occur?**

During the Project and In the Future

## Activities and Milestones

### Activity 1: Incorporate Minnesota’s citizen involvement in the collection and identification of freshwater sponges.

**Activity Budget:** \$208,500

#### Activity Description:

Numerous rivers and lakes in Minnesota remain to be explored for freshwater sponges. Collections will continue with the additional focus of citizen scientist involvement. Town hall meetings will be held at strategic locations across the state to explain the freshwater sponge project, and encourage citizen scientist participation. Using developed specimen collection packets, citizens will be able to collect sponge samples and mail them to UMC for taxonomic (research addendum section 4.1.3) and chemical (research addendum sections 4.2.2 and 4.2.3) analyses. . Dr. Robert Blair, Professor & Extension Specialist, University of Minnesota Twin Cities will assist the team by arranging town hall meetings and coordinating with the University of Minnesota’s Extension Master Naturalist program. Faculty/researchers will also travel to schools in Northwest Minnesota to engage students and teachers in STEM activities related to freshwater sponges. Freshwater sponge-focused activities will be designed to get students interested in scientific inquiry and stimulate participation in local and regional science fairs.

#### Activity Milestones:

Description	Approximate Completion Date
Develop information/collection packets to send to the public for collection of sponges	March 31, 2022
Collection of sponges from lakes/rivers within each major basin/watershed in MN.	October 31, 2023
Perform outreach activities using town halls, schools, etc. to stimulate sponge collection and STEM education	June 30, 2024

### Activity 2: Identify compounds produced by freshwater sponges that could be used to combat the spread of aquatic invasive species

**Activity Budget:** \$136,000

#### Activity Description:

Freshwater sponges, as well as water and sediment samples, will be collected. Sponges will be freeze-dried prior to chemical extractions. Organic components of these samples will be analyzed using chromatographic techniques (e.g., GC-MS with NIST library, LC-MS). Inorganic components will be analyzed using chromatographic (e.g., IC) and spectroscopic (e.g., ICP-MS) techniques.

All sponge extracts will be tested for their potential antifouling activity using zebra mussel attachment and in vitro assays. Compounds of interest will be identified using GC-MS and LC-MS and where possible, isolated in order to fully test their efficacy. The efficacy of readily available long chain amides such as oleamide against zebra mussels will be tested using compounds obtained from chemical suppliers.

#### Activity Milestones:

Description	Approximate Completion Date
Obtain permissions from MN DNR and collect Zebra Mussels for performing reattachment assay	December 31, 2021
Perform Zebra Mussel reattachment assay with commercially available long chain amides	June 30, 2022
Examine the chemical ecology of freshwater sponges	June 30, 2024
Isolate antifouling compounds and perform biological assays to assess the antifouling ability of sponge extracts	June 30, 2024

### Activity 3: Stimulate STEM education for students in Minnesota

**Activity Budget:** \$55,500

**Activity Description:**

During phase 1 of the freshwater sponge project, two community colleges and a few high schools reached out to us and we have been training their students in the study of sponges. UMC annually conducts the Western Regional Science Fair and school children from the ten surrounding counties compete in the Fair. We will collaborate with interested school districts and high school science teachers to stimulate STEM education. This is in addition to training our own undergraduate students.

**Activity Milestones:**

Description	Approximate Completion Date
Stimulate STEM-related experiences to high school students, partner with additional community colleges, and train undergraduates	June 30, 2024
Assess the impact of STEM-related opportunities at all levels of education	June 30, 2024

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Anthony Schroeder	University of Minnesota Crookston	Co-Principal Investigator	Yes
Timothy Dudley	University of Minnesota Crookston	Co-Principal Investigator	Yes

## Dissemination

**Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.**

The results of this project will be inherently disseminated to citizens in Minnesota who chose to participate in the project. The results of the project will also be presented at the town hall meetings to provide attendees with information about how their involvement in the project will continue to provide information about organism diversity in Minnesota. The incorporation of this project into the Zooniverse platform will also allow citizens to be involved in the project and be updated with the results of the project. Citizens will be able to utilize the Zooniverse platform at <https://www.zooniverse.org>. A listserv will be set up via the University of Minnesota Crookston communications office at <https://www.crk.umn.edu/units/university-relations>. Additional information can be obtained by visiting the freshwater sponges' website at <https://freshwatersponges.crk.umn.edu>.

Undergraduate students will still be heavily involved in this research, despite the importance of including Minnesota's citizens, the students will be presenting their results via poster and oral presentations at any number of local, regional, and national conferences and symposia. UMC hosts an undergraduate research day each year in which students discuss the research projects they work on with their peers and other faculty. Recently, UMC students have presented their research findings at both regional and national meetings (e.g., American Chemical Society, National Council of Undergraduate Research). Similarly, UMC faculty have been presenting at many of these and other conferences (e.g., Society of Environmental Toxicology and Chemistry). We plan to continue our attendance at these conferences in order to disseminate our findings for this project.

We have also been in contact with various state agencies as a result of the initial sponge project. We will continue to work closely with the Minnesota Department of Natural Resources (DNR) and Pollution Control Agency. The DNR will be especially interested in any anti-fouling compounds that we identified from the freshwater sponges. We have also contacted Nicholas Phelps at the University of Minnesota Aquatic Invasive Species Center about the project. He mentioned that this research could be a potential partner project and we are interested in working with the center to help disseminate the results of this project.

Lastly, we have tried to publicize the project through various media outlets. We will continue to try to do this as well as this is another way to let the people of Minnesota know about the project and how it is contributing to Minnesota's natural resources because of funding through the ENRTF.

## Long-Term Implementation and Funding

**Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?**

The diversity and distribution data will be shared with MN DNR annually through the project completion. The findings and results will be published in scientific journals. The project may generate new scientific questions. Further research if warranted will be funded by seeking grants from multiple resources including but not limited to the University of Minnesota and federal agencies.

### Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Mapping Taxonomy and Environmental Toxicology of Minnesota Freshwater Sponges	M.L. 2017, Chp. 96, Sec. 2, Subd. 03m	\$258,000



## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>Personnel</b>										
Venugopal Mukku		Project Manager 11% FTE in years 1 and 2, 22% in year 3. 1 month salary in year 1 and 2 and 2 months salary in year 3. Supervise students, coordinate the project, compile and file reports, disseminate results.			36.5%	0.33		\$43,032	-	-
Timothy Dudley		Investigator, 11% FTE in years 1 and 2, 22% in year 3. 1 month of salary for first two years and 2 months for third year. Supervise students, develop curricula, compile and file reports, and organize the dissemination of results.			36.5%	0.33		\$46,300	-	-
Anthony Schroeder		Co-Principal Investigator, 22% FTE in year 1 and 11% in years 2 and 3. 2 months salary in year 1 and 1 month salary in years 2 and 3. Responsible for activity 3.			36.5%	0.33		\$38,973	-	-
Research Fellow		work with PIs on all activities			36.8%	2.25		\$124,300	-	-
3 Undergraduate students		Summer research and field work			0%	3		\$58,418	-	-
3 undergraduate students		Lab/field work on activities 1, 2 and 3 during academic year			0%	3		\$24,000	-	-
							<b>Sub Total</b>	<b>\$335,023</b>	<b>\$256,212</b>	<b>\$78,811</b>
<b>Contracts and Services</b>										
Shimadzu	Internal services or fees (uncommon)	with the constant use of GCMS on the project, we need a service contract. (\$6,600/year). Shimadzu technicians will perform annual preventive maintenance and will be on call for any service-related issues.				2		\$13,200	\$12,600	\$600

TBD	Internal services or fees (uncommon)	For performing analysis of sponge extracts and water sample (LC-MS/MS, ICP-MS), (\$20/sample). These analyses will be performed either at the University of Minnesota Twin Cities or the University of North Dakota.				3		\$6,000	\$2,805	\$3,195
							<b>Sub Total</b>	<b>\$19,200</b>	<b>\$15,405</b>	<b>\$3,795</b>
<b>Equipment, Tools, and Supplies</b>										
	Tools and Supplies	Tubes, bags, supplies (100 sponge samples and 100 water samples) by investigators	To store collected sponge specimens by investigators at \$22.5/sample					\$5,500	\$3,500	\$2,000
	Tools and Supplies	Tubes, mailing boxes, reagents for fixing sponges (200 samples)	To make kits for sending to citizen scientists for collecting samples (at \$45/sample)					\$8,100	\$7,263	\$837
	Tools and Supplies	General chromatography supplies such as GCMS vials, columns, reagents, solvents	For performing analytical chemistry work					\$7,100	\$6,603	\$497
	Tools and Supplies	Culturing reagents and other consumables for approximately 300 assays	For performing Zebra Mussel assays (at \$15/assay)					\$4,500	-	\$4,500
	Tools and Supplies	Primers Big Dye Reagent, tubes, service costs	For DNA sequencing (approximately 300 samples) at \$15/sample					\$3,750	\$3,742	\$8
	Tools and Supplies	Chemicals	For sponge morphology experiments and extracting the sponges with organic solvents (at \$15/sample)					\$3,500	\$346	\$3,154
							<b>Sub Total</b>	<b>\$32,450</b>	<b>\$21,454</b>	<b>\$10,996</b>
<b>Capital Expenditures</b>										
							<b>Sub Total</b>	-	-	-
<b>Acquisitions and Stewardship</b>										

							<b>Sub Total</b>	-	-	-
<b>Travel In Minnesota</b>										
	Miles/ Meals/ Lodging	Miles plus meals	Covers costs for field trips for collecting sponges, renting University vehicles and meals					\$6,600	\$3,757	\$2,843
	Miles/ Meals/ Lodging	Lodging	Overnight stays on multi-day field trips and town hall meetings					\$2,000	\$523	\$1,477
							<b>Sub Total</b>	<b>\$8,600</b>	<b>\$4,280</b>	<b>\$4,320</b>
<b>Travel Outside Minnesota</b>										
							<b>Sub Total</b>	-	-	-
<b>Printing and Publication</b>										
	Printing	Infographics and documents	For distribution to citizen scientists and high school students					\$2,227	\$94	\$2,133
							<b>Sub Total</b>	<b>\$2,227</b>	<b>\$94</b>	<b>\$2,133</b>
<b>Other Expenses</b>										
		Shipping costs	For sending prepaid collection kits to citizens and for sending samples for chemical and DNA analysis	X				\$2,500	\$845	\$1,655
							<b>Sub Total</b>	<b>\$2,500</b>	<b>\$845</b>	<b>\$1,655</b>
							<b>Grand Total</b>	<b>\$400,000</b>	<b>\$298,290</b>	<b>\$101,710</b>

### Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Other Expenses		Shipping costs	Since the project involves citizen scientists collecting sponge samples from around Minnesota, the PIs will need to send collection kits with instructions to various locations around Minnesota. DNA and chemical analyses must be performed at remote locations and the samples will be shipped to those locations.

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
<b>State</b>						
In-Kind	Indirect costs (waived)	These are F&A Indirect costs which are waived	Secured	\$212,000	-	\$212,000
			<b>State Sub Total</b>	<b>\$212,000</b>	<b>-</b>	<b>\$212,000</b>
<b>Non-State</b>						
			<b>Non State Sub Total</b>	<b>-</b>	<b>-</b>	<b>-</b>
			<b>Funds Total</b>	<b>\$212,000</b>	<b>-</b>	<b>\$212,000</b>

## Attachments

### Required Attachments

#### *Visual Component*

File: [8497c88e-c4b.pdf](#)

#### *Alternate Text for Visual Component*

The graphic has the title of the project (Freshwater Sponges and AIS: Engaging Citizen Scientists) at the top center. The page contains 4 pictures. Clockwise they are a lake in which perhaps citizen scientists notice a sponge and inform the researchers followed by a meeting hall where citizen scientists listen to the investigators and Master Naturalists at three o' clock. At six o' clock, there is a picture of a zebra mussel, an invasive species and at nine o' clock, a picture of greenish spo...

### Supplemental Attachments

#### *Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other*

Title	File
Background Check Certification	<a href="#">86b14c96-a56.pdf</a>
Activity 1 Update April 2022	<a href="#">6bc81d0b-8bd.docx</a>
Activity 2 Update April 2022	<a href="#">fb250182-fa7.docx</a>
Freshwater Sponge and Citizen Science Slides	<a href="#">32ce0780-dc6.pptx</a>
Sponge Kit Information	<a href="#">23740451-511.pdf</a>
Minnesota Freshwater Sponges	<a href="#">c133d97c-37c.pdf</a>

### Difference between Proposal and Work Plan

#### *Describe changes from Proposal to Work Plan Stage*

The budget has been reduced to the recommended amount. Due dates for various activities were updated.

## Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

**Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?**

N/A

**Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?**

Yes, I understand the UMN Policy on travel applies.

**Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?**

No

**Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?**

N/A

**Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?**

N/A

**Does your project include original, hypothesis-driven research?**

Yes

**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration

## Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	<ul style="list-style-type: none"> <li>• Budget - Personnel</li> </ul>	<p>We intended to hire one of our colleagues who is already involved with the project as a postdoctoral fellow. But due to the COVID-19 related delays and shutdowns of labs, he is yet to submit the thesis. So for this year, we would like to include him in the project as a Research Fellow.</p>	July 12, 2022	Yes	July 12, 2022



# Status Update Reporting

## Final Status Update August 14, 2024

**Date Submitted:** October 23, 2024

**Date Approved:** November 27, 2024

### Overall Update

The project has made significant strides toward achieving the originally stated outcomes, particularly in increasing public awareness and engagement with Minnesota's freshwater sponges. Over the course of the project, 194 sponge collection kits were distributed to citizens across the state, leading to 41 kits being returned with samples. These efforts have resulted in the identification of eight distinct species of freshwater sponges, including three that were not previously reported in Minnesota. While some challenges were encountered, such as the low return rate of kits and incomplete molecular genetics identification, the project's outreach and education components were successful. Additionally, chemical analyses of water samples are ongoing, aiming to correlate specific environmental factors with sponge presence. The cumulative progress toward project outcomes has been positive, and the data collected will contribute valuable insights into Minnesota's aquatic ecosystems. Detailed analysis of the chemical composition of sponges collected in MN (by GC-MS) led to a reinforced understanding of the chemical ecology of freshwater sponges. One interesting metabolite that is present in almost 50 sponge specimens is 1,3,5 triphenylcyclohexane. This is a styrene trimer and is sometimes detected in packaged foods. However, this compound was not reported as a sponge metabolite in literature.

### Activity 1

Activity 1 focused on involving Minnesota citizens in the collection and identification of freshwater sponges. During the grant period, 194 sponge collection kits were distributed, with 41 kits returned containing samples. These efforts led to the identification of eight species, three of which were previously unreported in the state. Outreach efforts included attending county fairs, where citizens were educated about freshwater sponges and provided with collection kits. In 2023, a freshwater sponge collection site was established on CitSci.org, enhancing data submission and image sharing by participants. The project team also collected 67 additional samples, contributing to a total of 106 samples analyzed for spicule morphology and DNA sequencing. Despite some challenges, the activity successfully engaged citizens and advanced scientific knowledge of Minnesota's freshwater sponges.

*(This activity marked as complete as of this status update)*

### Activity 2

Activity 2 focused on the field collection and analysis of freshwater sponges. The team collected 67 sponge samples, which were analyzed for spicule morphology and DNA sequencing which led to the confident identification of eight freshwater sponge species, including three new records for Minnesota. The collected data is being used to correlate environmental factors, such as silica and iron content in water, with sponge presence and growth. Although challenges remain, the project's achievements in this activity have expanded the understanding of Minnesota's aquatic ecosystems. On the chemical ecology side, 1,3,5-triphenylcyclohexane, a common impurity in polystyrene food containers (U.S National Library of Medicine, n.d) is found in almost 50 sponges, which is an intriguing discovery.

Two zebra mussels were collected with a freshwater sponge sample in July 2023 and were stored in a one-liter bottle filled at room temperature with water from the sponge collection site. Additional water was added periodically to maintain their environment. Unfortunately, the mussels did not survive beyond 3-5 days. The deceased zebra mussels and water were autoclaved and discarded.

Multiple permit requests to our regional DNR office, DNR Invasive Species Specialist, and the UMN MAISRC were unanswered probably due to COVID-related contingencies.

*(This activity marked as complete as of this status update)*

### **Activity 3**

Activity 3 was dedicated to the chemical analysis of water samples collected in conjunction with sponge samples. The research team conducted standard chemical analyses, including pH, conductivity, and hardness, as well as the measurement of silica and iron content, which are believed to influence sponge development. Although the molecular genetics identification of sponges was not fully completed as intended, the ongoing analysis of chemical data is expected to provide insights into the relationship between water chemistry and sponge presence. The activity has been largely successful in advancing the project's objectives, contributing valuable data to the study of Minnesota's freshwater ecosystems.

*(This activity marked as complete as of this status update)*

### **Dissemination**

The dissemination of project results has focused on sharing findings through various channels. The team has engaged with the public through county fairs, where citizens were educated about freshwater sponges and provided with collection kits. Additionally, a freshwater sponge collection site was created on CitSci.org, allowing for broader citizen participation and data sharing. Data and information were also shared with the Extension Office and MN DNR. The project team is preparing to share results through scientific publications and presentations at relevant conferences. While some dissemination activities are still in progress, the efforts to date have successfully raised awareness and contributed to the scientific community's understanding of freshwater sponges in Minnesota.

# Status Update Reporting

## Status Update April 1, 2024

**Date Submitted:** October 23, 2024

**Date Approved:** November 27, 2024

### Overall Update

Steady progress is being made on sponge molecular analyses and chemical analyses. Citizen science aspect of the project is being concluded with a few more representations at science fairs over the summer.

### Activity 1

No more collections were done during the reporting period. Continued analysis of citizen provided samples and those collected by the PIs is in progress. Extensive GC-MS data is generated and is being analyzed. An update was provided by Karl Anderson at the Fisheries Summit conducted by MN DNR in April 2024. MN DNR is looking to emulate aspects of our process to disseminate their findings.

### Activity 2

The iron concentration of the samples collected (22) were all strip tested and 14 were tested for iron using spectrophotometric methods. We are continuing to look for a better method to determine the silica concentration in the samples. We reexamined the GC-MS profiles of 60 sponge extracts and the results will be shared at the time of the final report. Twelve samples were selected for confirming analysis. These samples will be sent to the Twin Cities metabolomics center for confirming the tentative identities of compounds made using the NIST library.

Two undergraduate students were trained in analyzing the GC-MS data and retrieving relevant literature. One student was trained in performing sponge extractions. Four more students had hands on experience in molecular techniques.

### Activity 3

We also had a group of AP Biology and AP Chemistry students from Stephen-Argyle come to campus (April 8) and we went through some of the steps of how to process a water sample (i.e. hardness of water titrations, iron concentrations, and silica concentrations). These students also visualized sponge samples underneath a microscope and performed a simple digestion of sponge using bleach.

We plan to start searching for sponge earlier this summer (mid-May) due to the warm winter and early thaw. We also plan to attend 14 county fairs this summer where we will again pass out sponge collection kits to citizens.

### Dissemination

An update was provided by Karl Anderson at the Fisheries Summit conducted by MN DNR in April 2024. MN DNR is looking to emulate aspects of our process to disseminate their findings.

# Status Update Reporting

## Status Update October 1, 2023

**Date Submitted:** October 23, 2023

**Date Approved:** November 9, 2023

### Overall Update

Freshwater Sponge Project was discussed with various legislative bodies during the American Society for Microbiology's Hill Day in Washington D.C. on 9.28.2023. MN representatives expressed additional interest in the project. In addition, the project was widely disseminated at a number of county fairs during the summer months. The PIs, students, made a number of field trips to collect more sponges. A few specimens were also collected by citizen scientists and sent to Crookston. Permissions to collect sponges and zebra mussels were sought and progress is being made on that front as well.

### Activity 1

Outreach consisted of visiting 11 county fairs in Minnesota: Clearwater, Kittson, Mahnommen, Marshall, Norman, Pennington, Polk, Rock, Roseau, Stevens, and Wadena. Rock and Stevens, were by invitation of the University of Minnesota to be part of their Driven to Discover (D2D) research exhibition. During these events, we discussed with the public our research involving freshwater sponges and asked citizens if they would be willing to locate and collect sponges for our study. We provided around 85 kits for citizens that were interested. We also handed out 15 kits to homeschool students in northwest Minnesota who were at UMC for a STEM day. A meeting with the MN DNR Fisheries Office is scheduled for October 17th in Sauk Rapids. Further meetings via Zoom are planned for November.

As part of many of these county fair visits, we also collected 50 sponges from 20 locations. We went on 14 trips.

### Activity 2

This summer we performed a number of chemical analyses on water samples collected from our sponge collection trips as well as those sent back to us by citizen scientists. This involved performing standard chemical analyses such as pH, conductivity, and hardness of water experiments. We have also been analyzing silica content in these water samples to try and correlate silica concentrations to presence and potentially development of sponges. We have also used simple test strips to measure other chemical species such as nitrates and phosphates, which may be present due to agricultural runoff. Zebra mussels were collected but died and were properly disposed.

The sponge samples that were collected have been processed for their various endpoints including frozen for spicule preparation, storage for DNA analysis, and freeze-dried for chemical analysis.

The imaging of the slides is ongoing. DNA has been extracted for approximately half of the samples collected this summer. DNA extractions will continue through the fall. The extracted DNA will be used for PCR and sequencing to identify species.

Chemical data via GCMS was reviewed. Samples were selected for further genetic sequencing. Quotes are currently being sought for sequencing work in addition to that done in house.

### Activity 3

We have 3 undergraduate students working on different aspects of the project. We also engaged a number of students from northwest Minnesota attending multiple 4-H camps at Lake Bronson. During these visits we showed students how to locate and collect freshwater sponges as well as how we do some preliminary processing and identification of sponge species.

**Dissemination**

Freshwater Sponge Project was discussed with various legislative bodies during the American Society for Microbiology's Hill Day in Washington D.C. on 9.28.2023. MN representatives expressed additional interest in the project.

MN LCCMR was the topic of discussion during several meetings. Discussions beyond the sponge project occurred. -Great educational experience for MN legislators not typically involved with state-funded projects.

A meeting with the MN DNR Fisheries Office is scheduled for October 17th in Sauk Rapids.

We are in talks with Britt Marie Fosberg, UMN Extension, regarding conducting Zoom sessions for Master Naturalists.

We are planning a manuscript once we confirm the species level identification of the sponges collected so far. That manuscript will include the diversity, distribution, and chemistry of MN freshwater sponges.

# Status Update Reporting

## Status Update April 1, 2023

**Date Submitted:** April 23, 2023

**Date Approved:** April 27, 2023

### Overall Update

The two Co-PIs (Drs. Schroeder and Dudley) are engaging citizen scientists while one of them (Dr. Schroeder) is actively seeking to determine the taxonomy and diversity of sponges in Minnesota. Dr. Dudley also leads the inorganic analysis. Mr. Anderson who was hired as a research fellow is building partnerships with dive shops and engages with MN DNR in connection with the project. Dr. Mukku examined the chemistry of the sponge extracts.

### Activity 1

We have isolated DNA from the majority of the sponges that were collected and sent to us from citizens. We have used PCR to amplify the cytochrome oxidase I (COI) gene from the sponges. We have yet to perform the DNA sequencing to assist with the species identification. The DNA sequencing will be completed once DNA is isolated from the last few sponges samples and COI amplified.

We have been working to develop a place for citizens to more readily enter their data when collecting sponges. With the help of people at the University of Minnesota's Citizen Science Center, we are using the CitSci platform for entering data. We have started developing a project using this platform, and this upcoming summer, citizens will be able to enter their sponge collection and environmental data.

We are also developing a Zooniverse page to upload sponge spicule images. The images will be able to be accessed by citizens which will then use the platform to measure the length and width of the spicules. The spicule measurements and other morphological characteristics will allow the citizens to help us identify the species of the sponges that they collected for us.

### Activity 2

The chemical examination of all the sponge extracts is complete and the results were disseminated at a Faculty Research Showcase that was organized by the PI on Campus. The principal sterols being identified in sponges are Cholesterol, Cholesta-4,6-dien-3-ol, Cholesta-3,5-dien-7-one, Cholesta-4-en-3-one, Campesta-4-en-3-one, Stigmasta-4,7,22-trien-3-ol, Stigmasterone, Stigmasta-4-en-3-one, Stigmasta-5,22-dien-3-ol. These sterols fall into three categories, namely the C27 sterols (cholesterol and its derivatives), the C28 sterols (campesterol and its derivatives), and the C29 sterols (based on the stigmaterol skeleton). The C27 sterols make up more than 60% of the sterol mixtures found in sponges. Sterols were not found in all the sponges however. Some sponge extracts contain long chain alkanes. More in-depth study is needed to confirm the identity of these alkanes.

Dr. Dudley is the lead on the inorganic analysis (using ICP-MS, IC, and TOC) but that was paused due to the instrumentation being down.

All sponge extracts prepared thus far were cleaned and are now ready for biological analysis (such as antifouling assay). However, due to the extremely limited amounts of extracts (a few milligrams in certain cases), isolating individual compounds is not possible.

### Activity 3

The outreach portion of the project has focused primarily on planning activities for the summer of 2023. Between mid-June and mid-August we plan to attend eight county fairs in Northwest Minnesota and two more in Southwest

Minnesota in conjunction with the Driven to Discover (D2D) program through the University of Minnesota Extension. This is twice as many fairs as we attended the previous summer (5), with most fairs located in the bottom-half of the Lower Red River water basin. We have received positive feedback from the fairs we attended last year.

We will continue to engage fairgoers by bringing engaging activities for children (e.g., microscopes to look at sponges) and provide sponge collection kits for those interested in searching for sponges. Last summer we encountered a number of home school groups and were able to provide them STEM-based experiences on our campus during the 2022-23 school year. We are planning more sessions with these groups in the coming months that focus on freshwater sponge analysis and collections.

Mr. Anderson is planning to join two dives (Big LaSalle Lake and one of the mining pits) this summer to collect from places I'm told there are deeper sponges.

### **Dissemination**

We are working with the CitSci and Zooniverse to develop websites to upload spicule images so that our citizen scientists can participate in the identification of the sponges they collected. We have presented a poster at the Faculty Research Showcase on Crookston Campus that was organized by the PI. The poster is attached.

# Status Update Reporting

## Status Update October 1, 2022

**Date Submitted:** November 2, 2022

**Date Approved:** November 4, 2022

### Overall Update

We went to five county fairs to disseminate our research and to distribute sponge collection kits to interested citizens. A total of 81 kits were given out. We have received a few kits back with sponge samples. They are being studied to determine their taxonomy. The research team did not travel to collect sponges and the chemistry during the period was focused on cleaning up the extracts prepared earlier for GC-MS analysis. We will have completed the chemical analysis of all sponges collected thus far by the next report. Finally, the zebra mussel assay was not done so far. We hope to get the permit to collect zebra mussels and collect them next year.

### Activity 1

We continued to engage the public in our freshwater sponge project by attending a number of county fairs in Northwest Minnesota this summer. At each fair we set up a couple of tables where kids and adults could use microscopes to see the parts of a sponge (e.g., spicules, gemmules). Our discussions typically involved where we have found sponges, where we expect sponges to be found in the area, and how we identify sponges once collected. We received a large amount of positive feedback about this project and many families stopped by our booth at each fair. Some even took a sponge collection kit with them (or more than one) in an effort to help us locate and identify sponges in bodies of water we had not been to. We visited the Norman, Polk, Pennington, Marshall and Beltrami County fairs and distributed 81 collection kits. We received 6 collection kits back. Gemmules were extracted from the sponges and identified the gemmuloscleres from the gemmules. The DNA extraction is in process to get the genetic code of the species.

### Activity 2

Permissions to collect sponges was obtained in May 2022. A research fellow was hired in July 2022. No sponge samples were collected during summer due to scheduling issues. However, all the sponge extracts prepared earlier are cleaned up by three undergraduate students from January to May and from August to October. The process involved resuspending the initially prepared sponge extracts in dichloromethane and filtering them through PTFE syringe filters to make them suitable for GC-MS analysis. GC-MS analysis of some extracts revealed the presence of a series of steroids and some compounds related to phytol.

### Activity 3

As part of the new Center for Rural Education in Science and Technology (CREST) established at the University of Minnesota Crookston, we have begun interacting with schools in Northwest Minnesota to support STEM activities. Part of this is educating students about freshwater sponges and enlisting their help in locating and collecting sponges. We started this in the late spring on a visit to Warroad High School. We discussed potential projects involving freshwater sponges that students could engage in and hope to come up with an action plan during the 2022-23 school year.

We plan to introduce the freshwater sponge project to more schools through our CREST activities in the 2022-2023 school year. This project has a number of activities that are appropriate for students ranging from middle school to high school.

### Dissemination

As mentioned earlier, the project is being disseminated heavily at science fairs. We will also present at the upcoming National Conference on Undergraduate Research in April 2023. In addition, we are in touch with members of the DNR.



# Status Update Reporting

## Status Update April 1, 2022

**Date Submitted:** April 29, 2022

**Date Approved:** May 6, 2022

### Overall Update

We developed a kit that allows for citizens to collect freshwater sponges and also environmental data. We piloted the sponge kits with undergraduate students from the University of Minnesota on a collection trip to Cass Lake. Sponges and environmental data were collected by the students and they provided feedback on ways to improve the kit and for the directions on how to use the kit.

### Activity 1

The funding was finally approved in August 2021 which limited our ability to do collections during the summer months of 2021. We have also been trying to get a permit to collect sponges from various watersheds. There was some communication gap (regarding permitting) which has now been sorted.

In the meantime, we have developed the kits as specified in Activity 1, Milestone 1, and have also set up collaborations. We have not yet set up any town hall meetings. The detailed report which includes instructions for citizens to collect sponges with pictures is attached.

### Activity 2

We were unable to do field trips due to the timing of the approval and also the delay in getting permits. However, we have continued to work on the chemical examination of sponges collected in Phase 1. A couple of students spent the spring semester studying the chemical composition of sponges collected in Phase 1. Many sponge extracts contained sterols whereas a few contained algal metabolites such as neophytadiene. An Excel file with the preliminary identification of sterols from a few sponges is attached.

### Activity 3

We have been working with a few high schools to increase STEM-related experiences. The outreach and training activities are in addition to training our own undergraduate students. We were able to train two students as part of their organic chemistry laboratory in studying the chemical metabolites of sponges using GC-MS and NIST spectral libraries.

### Dissemination

So far we were able to disseminate the chemistry of sponges at the spring semester day on our campus (April 7)